REMARKS

I. Support for the Amendments

Claims 18 and 19 were withdrawn by the Examiner, and new claims 20-25 have been added. Claims 1-3, 6-12, and 15-25 are currently in the application.

Support for new claims 20-25 can be found throughout the original specification as filed, including the Examples. Additional support for new claims 20 and 21 can be found in the language of claims 1 and 9 as originally filed. Additional support for new claim 22 can be found in the language of claim 3 as originally filed. Additional support for new claims 23 and 24 can be found in the language of claims 10 and 17 as originally filed. Additional support for new claim 25 can be found in the language of claim 12 as originally filed.

More particularly, additional support for new claims 20 and 21 can be found, e.g., on p. 4, ll. 5-17; from p. 4, l. 28, to p. 5, l. 3; from p. 6, l. 29, to p. 7, l. 9; on p. 10, ll. 1-19; page 11, l. 1, to page 12, l. 2; and in the Examples. Additional support for new claims 23 and 24 can be found, e.g., on p. 5, ll. 18-23; and on p. 10, ll. 21-30; page 11, l. 1, to page 12, l. 2. Additional support for new claims 22 and 25 can be found, e.g., on p. 4, ll. 25-26; on p. 6, ll. 1-26; on p. 8, ll. 5-13; and in the Examples.

II. Status of the Claims

Claims 1-17 were originally filed with the application and were subject to a restriction requirement. Claims 1, 6-10, and 15-17 were stated by the Examiner to be generic. In the Response to the Election/Restriction Requirement, Applicants elected Group II species vascular endothelial growth factor (VEGF). The Examiner requested Applicants

to identify claims readable on this species. Applicants noted that "[t]hese generic claims and claims dependent thereon are readable on Group II" species.

In the previously filed Amendment (mailed October 7, 2004), claims 4, 5, 13, and 14 were canceled without prejudice to the filing of one or more divisional applications, and claims 18 and 19 were added.

Claims 1-3, 6-12, and 15-25 are currently in the application, with claims 1 and 10 being the independent claims. Claims 18 and 19 have been withdrawn by the Examiner, and new claims 20-25 have been added.

III. The Information Disclosure Statement Has Been Acknowledged

The Examiner has acknowledged the reference cited in the Supplemental Information Disclosure Statement mailed with the previous Amendment on 7 October 2004. Applicants thank the Examiner for acknowledging the reference.

IV. The Drawings Have Been Accepted

Applicants thank the Examiner for accepting the Drawings.

V. Rejection of Claims 1-3, 6-12, and 15-17 under 35 U.S.C. §103(a) over Sugihara, in view of Buttke and Breier, is Traversed

The Examiner has continued to reject claims 1-3, 6-12, and 15-17 under 35 U.S.C. §103(a) "as being unpatentable over Sugihara et al., in view of Buttke et al. and Breier et al." We disagree.

The Patent Office first discusses Applicants' previous Amendment and then alleges:

> These arguments have been fully considered but deemed unpersuasive. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981*); In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). The examiner agrees that a MTS assay measures cell viability and thymidine incorporation assay measures cell proliferation which are two different parameter. However, one ordinary skilled in the art would recognize that **measuring one parameter does not excludes one from the measuring other parameter(s)**. Buttke provides sufficient reasons for combining the teaching because it teaches that it is of particular interest to compare MTS production with thymidine uptake in cell culture. In addition, **whether the plasmid is transfected transiently or stably does not impart a structural difference for said plasmid**. The endothelial cell proliferation mitogen produced by the plasmid would have been the same, thus **the conditional medium from such cultured cells are the same**. As such, one of ordinary skilled in the art has sufficient reason to combined the teaching of Sugihara and Buttke and reach the present invention of testing the mitogenic activity of the endothelial cell mitogen encoded by a test plasmid and measure endothelial cell viability when such cells are cultured with the conditioned media that comprises said mitogen. Breier et al. simply teach that Cos-1 cells are capable of being used to express VEGF, a endothelial cell mitogen. Buttke neither teaches away from Sugihara nor Breier because it provides sufficient motivation to combine the teaching of the references. Therefore, the claimed invention is obvious in view of the combined teaching of Sugihara, Buttke and Breier. This rejection is maintained. [Pp. 3-4; italics in original; other emphasis added.]

Applicants respectfully disagree with these comments and continue to traverse the obviousness rejection. Applicants submit that the previous arguments were not made simply to attack the references individually, but to point out the absence of suggestion within the references to combine these references.

With respect to Sugihara and as noted in the Amendments mailed on December 23, 2003, and October 7, 2004, Applicants and the Patent Office are in agreement that the transfection assay method described therein would appear to involve stable, rather than transient, transfection and that Sugihara does not teach using Cos-1 cell line as host cells expressing the endothelial mitogen protein. The Patent Office's contention that, "whether the plasmid is transfected transiently or stably does not impart a structural difference for said plasmid," is confusing given that the present claims are method claims. The Patent Office attempts to argue that the "endothelial cell proliferation mitogen produced by the plasmid would have been the same, thus the conditional medium from such cultured cells are the same," but this statement ignores the quantitative difference between the two types of media, as discussed in the Declaration, signed by inventor Marianne Kearney.

As noted in the Declaration, submitted herewith, stable transfection techniques typically select only for the transfected cells. Transiently transfected cells are typically mixed populations of cells, including some cells that are not transfected. As a result, a transiently transfected culture would be expected to yield less protein (i.e., a less concentrated conditioned media) than a culture stably transfected with the same plasmid.

The current invention utilizes analysis of cell viability as a tool to confirm sufficient protein production by the gene construct , because the proposed gene product(VEGF proteins) is known to act as a survival factor (increasing cell viability) for specific cell types such as Human Umbillical Vein Endothelial Cells.
Thus, the present invention represents an improvement over the art.

Previously, Applicants also noted that **Sugihara describes a cell mitogenic assay using incorporation of ^{3}H-thymidine during the cell cycle as a means of measuring cell proliferation**. In contrast, **the present invention provides a method for testing the**

survival of cells, that is, their ability to overcome cell death, **as measured by a cell viability assay**. These **traits are quite different**, because viable cells are not necessarily undergoing mitosis. **Sugihara does not teach measuring cell survival by tetrazolium (MTS)/formazan assay** or by any other assay, because **Sugihara does not teach measuring of cell survival at all.**

With respect to Buttke, as noted by Applicants previously, this reference teaches an MTS/formazan assay (see page 12 of the specification). Applicants submit, however, that **Buttke repeatedly distinguishes between the use of the ^{3}H-thymidine assay to measure cell proliferation and the use of the MTS/formazan assay to measure cell viability**. Moreover, Buttke repeatedly emphasizes **the use of both assays to distinguish between cell proliferation and cell viability**, such as those described on page 238 with the FDC-P1 cell line.

The Patent Office alleges that Applicants are attempting to argue the references individually, but this is not the case. Rather, Applicants are pointing out that neither reference suggests use of the other and that the references actually teach away from each other.

Because **the cell proliferation assay of Sugihara and the cell viability assay of Buttke are measuring two different parameters, Buttke cannot supply the deficiencies of Sugihara**. There is **no motivation** for one of skill in the art **to use the stable transfection technique and cell proliferation assay** of Sugihara **to perform the transient transfection and cell viability assay** of the present invention. The cell proliferation assay described in Sugihara does not necessarily provide a measurement of cell viability as provided according to the present invention or as provided in Buttke. Moreover, there **is no suggestion in Sugihara** that measurement of cell viability, as opposed to cell proliferation, would be desirable.

Likewise, Buttke not only fails to remedy the deficiencies of Sugihara, but there is **no suggestion in Buttke that measurement of cell proliferation would necessarily be interchangeable with measurement of cell viability**. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides **an example**, described above, in which **cell proliferation ceases while cell viability continues**.

In essence, therefore, **Buttke teaches away from Sugihara** by emphasizing a marked preference for **the need for both tests** as a means of **measuring and comparing two different cell parameters**, including an example in which **the results of the two assays differed precisely because they were measuring two different parameters**. As a result, **any technical advantages** of the MTS/formazan assay over the ^{3}H-thymidine assay are **essentially irrelevant**, because the assays are **measuring two different cell parameters**.

Finally, Breier cannot supply the deficiencies of Buttke and Sugihara. The Patent Office alleges that "Breier et al. teach that conditioned media following transient transfection of expression vector comprising VEGF cDNA to Cos-1 cells are collected and assayed for mitogenic activity on bovine aortic endothelial cells (see page 524, 2nd col., 2nd paragraph, and page 522, Material and Methods, 1st paragraph)," but the passages cited by the Patent Office do not discuss cell survival.

As discussed at length in the Amendment mailed on October 7, 2004, 1) Breier equates "mitogenic effect" with "stimulating the proliferation of endothelial cells" and 2) Breier's assay, as described within the four corners of the Breier reference, merely refers to counting cells "in a Coulter counter" to measure "proliferation of endothelial cells."

Similar to Sugihara, Breier **describes a cell mitogenic assay using a Coulter counter to count cells as a means of measuring cell proliferation**. In contrast, **the present invention provides a method for testing the survival of cells**, that is, their ability to overcome cell death, **as measured by a cell viability assay**. These **traits are quite different**, because viable cells are not necessarily undergoing mitosis. Breier never distinguishes between cells at different stages of mitosis. **A mere cell count cannot distinguish between the two parameters of cell viability and cell proliferation. Breier does not teach measuring cell survival by simply counting the number of cells** or by any other assay, because **Breier does not teach measuring of cell survival at all**.

Buttke uses the **^{3}H-thymidine assay, rather than the Coulter counter, to measure cell proliferation**. Buttke not only fails to remedy the deficiencies of Breier, but there is **no suggestion in Buttke that measurement of cell proliferation would necessarily be interchangeable with measurement of cell viability**. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides **an example**, described above, in which **cell proliferation ceases while cell viability continues**.

Again, the Patent Office alleges that Applicants are attempting to argue the references individually, but this is not the case. Rather, Applicants are pointing out that neither reference suggests use of the other and that the references actually teach away from each other.

In essence, similar to Sugihara, therefore, **Buttke teaches away from Breier** by emphasizing a marked preference for **the need for measuring and comparing two different cell parameters**.

Moreover, there is no suggestion in Breier to combine the teachings of Breier with those of Sugihara and/or Buttke. Like Sugihara, Breier measures cell proliferation – not cell

viability. Breier does not distinguish between the two parameters. As a result, the use of COS-1 cells in the Breier assay is irrelevant, because the parameters being measured are different.

Applicants respectfully submit that the present claims 1-3, 6-12, and 15-17 fulfill the requirements of 35 U.S.C.§103(a) and request the Examiner's reconsideration of these claims accordingly.

VI. The Election/Restriction Requirement

The Examiner has withdrawn claims 18 and 19, which were added in the Amendment mailed October 7, 2004. The Patent Office alleges:

> Claims 18 and 19 are drawn to a method of preparing a plasmid producing biologically active endothelial cell mitogen protein. It is a different invention from the originally elected invention, which is directed to a method for testing a plasmid containing a gene encoding for an endothelial cell mitogen for the ability to produce a biologically active mitogen protein. Each method comprises different steps which does not render obvious of each other.
>
> Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 18 and 19 are withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03. [Pp. 4-5.]

Applicants traverse the restriction and withdrawal of claims 18 and 19. The search and examination of claims 18 and 19 would significantly overlap that of claims 1-3, 6-12, and 15-17. Applicants respectfully request the Examiner to reconsider the restriction requirement.

VII. Conclusion

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

Applicants hereby request a three-month extension of time for the Amendment and accompanying materials and hereby submit the requisite fee accordingly. If a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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